Author's response to reviews

Title: Clinical implications of thymidylate synthetase, dihydropyrimidine dehydrogenase and orotate phosphoribosyl transferase activity levels in colorectal carcinoma following radical resection and administration of adjuvant 5-FU chemotherapy

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Author's response to reviews: see over
Dear Dr Annabel Phillips,

Thank you for your letter of 02/08/2007. The comments of reviewers have been helpful in allowing us to revise our manuscript. We checked some mistakes and have attempted to address the questions raised by reviewers according to the following. We are enclosing the revised manuscript entitled "Clinical implications of thymidylate synthetase, dihydrofolate reductase and orotate phosphoribosyl transferase activity levels in colorectal carcinoma following radical resection and administration of adjuvant 5-FU chemotherapy" for consideration as a publication in *BMC CANCER*.

Thank you for your consideration of the revised version.
We appreciate your review of this work.

Sincerely,

Masashi Ishikawa MD Ph.D.
Department of Surgery
Tokushima RedCross Hospital
Reviewer: Carlo Barone

General

1) Methods for measurement of enzymes are very important. Therefore we added [The controversial conclusions about the predictive role of TS, DPD and OPRT in the neoadjuvant setting can also be explained by the different methodologies. The optimal method of assessing TS, DPD and OPRT expression is unclear at present. Methods used to determine TS, DPD and OPRT expression and assign expression status are immunohistochemistry (IHC), reverse transcriptase polymerase chain reaction (RTPCR) and enzyme assay. The commonest technique used to determine these enzymes expression for survival analysis was IHC. However, we chose enzyme assay because this method are simple and cost-effective although dichotomization of enzyme assay was by a threshold defined as that most likely to differentiate tumors by response, as seen in the RTPCR-based studies.] (P14,L10~)

2) As reviewer suggested, a number of studies regarding the role of TS and DPD were published. However, there are still controversial problems. Especially, further studies regarding prognostic role in DPD would be needed. We added [Taking into account these considerations, the role of DPD as a predictor of toxicity and tumor responsiveness for patients with DPD activity within the normal range awaits further clinical investigation in a prospective fashion before definitive conclusions.] (P13L26~)

3) Discussion were shortened, by deleting general statement of enzymes. We focused on relationship between enzymes activity and clinicopathologic factors, explanation about the lower level of OPRT in lymphatic metastases. As the number of patients are small, we used mild statement. [Thus, the OPRT activity ratio for tumor tissue/normal tissue significantly decreased as tumor stage increased in colorectal carcinoma. These findings indicate that it may be possible to predict lymphatic metastasis by determining OPRT activity in tumor tissue before surgery.] (P11L28~)

4) We rewrote as follows. [Patients with Dukes’ B corresponded with UICC stage II A (T3pN0,n=15) and UICC stage III A (T4pN0,n=7), and those with Dukes’ C corresponded with UICC IIIA (T3pN1,n=11) and UICC stage III B (T4pN1,n=7).] (P5L8~)

5) We changed as follows [These findings indicate that it may be possible to predict lymphatic metastasis by determining OPRT activity in tumor tissue before surgery.] (P12L2)
6) P13L16-18 was reported in Ref 11.

7) In conclusions, we used more cautious statement as follows. [TS and OPRT activity levels in tumor tissue may be important prognostic factors for survival in Dukes’ B and C colorectal cancers with radical operation and adjuvant chemotherapy of 5-FU. However, the conclusions are drawn from a limited retrospective study.] (P15L8~)

8) As reviewer pointed out, the small number of patients influenced conclusions. There we used mild statement somewhere.

9) We checked “predict” and “prognostic”.

10) Cutoff was taken as the median value. This was written in result. (P10L10)

11) The reference 1 was changed.

12) Page 5.lines4-7 was modified. [However, there have been a few reports investigating the relationship between the activity of these enzymes and duration survival in patients receiving radical operation combined with adjuvant 5-FU chemotherapy for colorectal carcinoma.] (P10L10)

13) P6,L16-18 were deleted.

14) Reaction was stopped by adding 10% active carbon.

15) One sentence was deleted.

16) P12,L13-24 were deleted.
Reviewer: Silke Lassmann

1) In patients with Dukes’ B and C, disease-free survival was also significantly better in the high
OPRT activity group and the low TS group, respectively. This finding was added in results.
Mistake in Table 2 was modified. (P10L13~)

2) In correlation of enzyme levels in tumor, it is important to rule out enzyme level in stromal
cells. However, it is a weak point in enzyme assay used in this study.

3) A multivariate analysis was done as follows and Table 3 was added. [Nine variables (TS, DPD,
OPRT, age, sex, tumor size, Histological type, lymphatic metastasis and tumor depth) were
analyzed using the Cox’s proportional hazards regression model to determine the factors
affecting the survival of colorectal cancer patients. Analyses showed TS activity (P=0.02) and
OPRT activity (P=0.05) to be significant variables to independently predict postoperative
survival. (Table 3)] (P10L18~)

3) This was modified as follows. [Tissue was taken from the tumor and adjacent tumor-free sites
( > 5 cm from tumor) of the resected sample, and immediately frozen in liquid nitrogen without
impairment of enzyme activities and stored at -80°C until use.] (P5L24~)

4) Statement of the description of OPRT activity was deleted partly and was shortened.

5) Cutoff was taken as the median value. This was written in result. (P10L10)

6) This sentence was deleted.

7) Discussion was shortened, by deleting general statement of enzymes and focused on
relationship between enzymes activity and clinicopathologic factors, and the role of enzymes as
prognostic factors.